IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

ATTORNEY DOCKET NO.: 076333-0240

Applicant: Robert H. Getzenberg

Title: BLADDER CANCER NUCLEAR MATRIX PROTEINS, POLYNUCLEOTIDE SEQUENCES ENCODING THEM, AND THEIR USE

Application No.: 09/866,927

Filing Date: May 30, 2001

Examiner: Larry Ronald Helms

Art Unit: 1642

SUPPLEMENTAL DECLARATION UNDER 37 C.F.R. §1.132

Commissioner for Patents
Box AF
Washington, D.C. 20231

Sir:

I, Robert H. Getzenberg, being duly warned, hereby declare and state:

1. That I am the named inventor of the subject matter disclosed in the United States application Serial No. 09/866,927 ("the application").

2. That this supplemental declaration of mine is to further clarify and explain that the BLCA-6 protein in the specification is the same as that of Getzenberg *et al.*, *Cancer Research* **56**:1690-94, 1996 (cited reference).

3. That the claimed BLCA-6 protein is above the 29 kDa molecular weight marker and, therefore, the MW is indeed about 31-kDa, and not 22-kDa.

4. That during the submission of the preprint of the cited reference, I received a communication dated December 20, 1995, from the Managing Editor of the Cancer Research journal, as well as comments from the reviewers (see Appendix A). On page 1 of the Dec. 20th letter, Reviewer #1 inquired about the MW discrepancy of the BLCA-

6 protein in Table 2 and Figures 1, 2B and 2C. In a letter dated January 3, 1996, I clarified this error by stating that the correct MW of BLCA-6 protein in Table 2 was indeed 31-kDa (see Appendix B). Unfortunately, this discrepancy was discovered more than a month after the filing of the provisional application but was never corrected in the provisional and parent applications or communicated to the office of the undersigned.

5. That I would like to stress that the correct molecular weight and pI of the claimed human BLCA-6 protein is about 31-kDa and 8.0 respectively, and that the correct molecular weight and pI are inherent properties of this protein. One of ordinary skill in the art, upon following the procedural steps for collecting the samples and performing the protein separation described in the application as filed, would inherently obtain a protein having a molecular weight and pI equal to about 31-kDa and 8.0, respectively.

6. That I have repeated this procedure following the disclosure in the specification and has obtained a protein having a MW and pI of about 31-kDa and 8.0, respectively (see Appendix C). Accordingly, the 31-kDa molecular weight and pI of 8.0 are both inherent properties of the BLCA-6 protein that result from the above-mentioned techniques. The inherent properties were shown in Figure 1B of the preprint and disclosed in the Getzenberg *et al.*, *Cancer Research* **56**:1690-94, 1996. Therefore, the claimed BLCA-6 protein in the specification the same as the BLCA-6 protein of Getzenberg *et al.* (1996).

7. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued therefrom.

Dated: _____

_____

Robert H. Getzenberg

APPENDIX A

Cancer Research

American Association for Cancer Research
Public Ledger Building • Suite 816
150 South Independence Mall West
Philadelphia, PA 19106-3483
Telephone: (215) 440-9300 • Fax: (215) 440-9354

December 20, 1995

Dr. Robert H. Getzenberg
Univ. of Pittsburgh Cancer Inst.
200 Lothrop Street
BST E-1056
Pittsburgh, PA 152132582

In reply please refer to MS No. CAN-2513-5
Bladder Cancer Associated Nuclear Matrix Proteins

Dear Dr. Getzenberg:

The Editors have completed examination of your above-referenced manuscript. We are pleased to inform you that it is acceptable for publication in Cancer Research, pending revision.

The principal concerns of the reviewers are additional information about the cell and tissue samples and presentation of the figures. Their comments are enclosed for your guidance in revising the paper.

The Editors ask that you shorten your presentation wherever possible to assist us in saving journal space. The reviewing team specifically notes that the Discussion must be abbreviated. Your cooperation will help us to ensure that every acceptable manuscript can be published.

We require that manuscripts that have been accepted in principle be revised and returned to us within four weeks. Delayed resubmissions may undergo additional review. We trust that you can accommodate this requirement, and we look forward to receiving your revised paper. With your resubmission, please include a covering letter to explain, point by point, how you have dealt with each comment.

We appreciate the opportunity to review your work.

Sincerely yours,

EDITORIAL BOARD

Margaret Foti, Ph.D.
Managing Editor

MF:lac
Enclosures

Cancer Research

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Carlo M. Croce, M.D., Editor-in-Chief
Margaret Foti, Managing Editor
Mary Anne Mannite, Assistant Managing Editor

Date: December 20, 1995

Author(s): R. H. Getzenberg, et al.

MS. No. CAN-2513-5

Your manuscript must adhere to the style of Cancer Research in the specific areas listed below. The Editor would greatly appreciate your cooperation in making the appropriate revisions before the manuscript is resubmitted.

WHEN YOU HAVE COMPLETED THE REVISIONS OF YOUR PAPER, PLEASE RECHECK THE REFERENCES TO ENSURE THAT THEY ARE ACCURATE AND THAT EACH ONE HAS BEEN CITED IN THE TEXT.

1. If you can supply your revised manuscript on disk, it will facilitate typesetting of the article, if accepted. Please follow the instructions on the attached guidelines for submitting disks.

2. On the hard copies of the manuscript, please note first citation of each table and/or illustration in the margin as well as in the text.

3. The panel label "A" is crooked in Figure 1A. Please straighten if possible.

Reviewer #1

This manuscript describes the 2-D gel analysis of the nuclear matrix protein composition of 17 matched tumor and normal samples from patients undergoing surgery for bladder cancer. Three human bladder cancer cell lines were included in these analyses. The authors identified six proteins present in all the tumor samples and three proteins unique to normal bladder tissue. I believe this study merits publication in Cancer Research. The following are minor criticisms that should be addressed.

1). In the Methods and Materials section (page 7) the authors describe the use of RNase A for removing chromatin structures but in the present study they use an RNase inhibitor. Why? Did they use RNase for this prep and if not, why not?
2). The authors indicate that the samples are matched. Is this age and sex?
3). Table 1 indicates that BLCA-6 has a molecular weight of 22 kD yet Figures 1B, 2B and 2C indicate a molecular weight above 30 kD.
3). Why are the cell line protein profiles more complex than both the healthy primary tissue and primary tumor profiles?
4). The Discussion is too long. First, summarize the results of the present study and edit some of the material concerning potential NMP cancer markers from the authors previous work. The authors should use the space to address how the identified potential cancer markers (i.e. BLCA1-6) can now be isolated and cloned. These silver-stained spots appear very faint. What experimental evidence or a priori justification can the authors offer that such low-abundant proteins will be detected in the serum or urine?

Reviewer #2

This study by the Getzenberg laboratory documents the use of high resoluti n 2D PAGE analysis in the cytopathologic screening for bladder carcinoma. The authors observe several uniqu proteins which are cancer-related by comparing the nuclear matrix protein composition of tissue-specimens from normal bladder and bladder tumors. The experimental approach is well-established and the authors have extensive experience in application of this technique. The conceptual background is extensively and clearly explained, the results are concisely presented, and the conclusions of the authors are appropriate. Based on the potential importance of nuclear matrix proteins in the diagnosis of cancer pathologies, this study is of general significance.

Minor points:

(1) There are several typographical errors which the authors ought to correct: p5, line 8: too many verbs in "...was able to be utilized to distinguish..."; ref 13: change capitals to lowercase in title; ref 23: Verheijen i.s.o. Verhiejen; ref 22: expression i.s.o. epxression.

(2) Correct use of Ref. 18: p7, line 5-6, "minimal disruption of ... structure (8,18)"; p7, line 7-8, "our previously published adaptation...(10,18)". Is the use of reference 18 appropriate in these cases?

Reviewer #3

This paper describes the nuclear matrix proteins of normal bladder tissue, bladder tumors and bladder tumor cell lines. Nuclear matrix proteins found in tumor but not normal, and vice versa were identified. Potentially, these tumor nuclear proteins could serve as markers for the cytopathological screening of bladder cancer.

The manuscript could be accepted for publication in Cancer Research once the points listed below have been addressed.

(1) Are the mitotic indices of the cells in normal and tumor tissue different? Could the differences in nuclear matrix proteins reflect changes in proliferation rates?

(2) I was not convinced that BLCA-1 is specific to tumors since this protein appears to be present in normal bladder nuclear matrix proteins. Is this protein lamin A? It would be useful to mark the placement of the lamins as was done in Partin et al (ref 11).

(3) I could not see the spots at the positions circled for BLNL-1, 2 and 3. I assume that the originals are better than the xeroxes.

(4) Fig. 1B is a representative 2D pattern of tumor nuclear matrix proteins. There are several proteins that are clearly more abundant in the tumor than in the normal bladder (e.g., the cluster of spots running next to BLCA-3 with similar pIs but greater molecular masses). A comment on the degree of variation in the 2D patterns of nuclear matrix proteins from normal bladder and tumors would be appreciated.

(5) The Discussion should be shortened.

APPENDIX B

January 3, 1996

Dr. Carlo M. Croce
Editor-in-Chief
Cancer Research
AACR
150 S. Independence Mall West
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Suite 816
Philadelphia, PA 19106-3483

MS No. CAN-2513-S

Dear Dr. Croce:

We appreciate your review of our manuscript entitled, "Bladder Cancer Associated Nuclear Matrix Proteins", for consideration for publication in *Cancer Research*. We have revised the manuscript by incorporating the modifications that were suggested by the reviewing team. We would therefore like to address each of the criticisms raised by the reviewers:

Reviewer #1

1. The methods in this manuscript utilize RNase inhibitors prior to the step in which RNase is added. It appears necessary to maintain the RNA in the nuclear matrix prior to this step to maintain the stability of the nuclear matrix. Therefore, the method is correct as written, RNase inhibitors were added initially and then RNase is utilized.

2. Normal and tumor tissue samples were collected from the same patients. They are therefore "matched" to mean that they come from the same patient. This has been clarified in the Methods and Materials section.

3. We apologize for this error. When the numbers were typed for Table 2, a mistake was made. The correct molecular weight for BLCA-5 is 32 kD and BLCA-6 is 31 kD.

4. While the figures chosen to represent the cell lines NMPs (Figure 2A-C), may appear to be more complex then the tissue samples, this is not the case. Admittedly, these figures do appear to have a number of darker staining proteins. Our experience with the cell lines indicates that they have a number of NMPs that appear more abundant than in the tumor tissue. In addition, these are only representative gels and some of the isolated samples result in gels of more similar staining patterns to the tumor samples. In directly answering the reviewer's question, we do not feel that the cell line protein profiles are more complex than both the healthy primary tissue and tumor profiles.

5. The Discussion was shortened as suggested. These changes include only summarizing the authors previous work and adding additional discussion on how the identified potential cancer markers can now be isolated and cloned.

Reviewer #2

All of the modifications of Reviewer #2 were made in the manuscript.

Reviewer #3

1. We have not directly quantitatively examined the mitotic indices of the normal and tumor tissues utilized in these studies. The experience of the pathologist involved in these experiments, indicates that while the mitotic indices of the bladder tumor tissues used in these studies may be slightly higher than the normal, these differences are not significant. While we currently do not have the quantitative data to rule out the possibility that these differences in

NMPs reflect changes in proliferation rates, we feel that this has only limited possiblity. Regardless, we feel confident that we have identified NMPs that are able to specifically differentiate normal bladder tissue from bladder tumors. As discussed in the manuscript, differential NMPs may reflect nuclear alterations including, DNA organization, gene expression, nuclear shape and these possibilities would even include the proliferation rate of the cell.

2. BLCA-1, while in proximity to the area where the Lamin A isoforms are found does not appear to be Lamin A. We will, of course, only know definitively, when we have the protein sequence data. We have modified Figure 1A, to include the placement of the lamins as was suggested. These lamin designations can then be applied to all of the other figures.

3. In the originals, BLNL-1, 2 and 3 are evident. They are relatively light spots and could certainly be lost in xerox copies.

4. While in this figure, there appear to be other abundant proteins in the tumor that are not present in the normal sample, several important points need to be addressed. First, we only selected proteins that were consistently found in the 17 tumors that we examined. Therefore, any proteins different in individual samples were discounted. In addition, we did not examine quantitative changes, and only looked for the presence/absence of a particular protein. Finally, silver staining of proteins is not quantitative. Therefore, while proteins that stain darker are often more abundant, this is not always the case. There is variation between samples. It is our estimation that 5-10% of the NMPs may vary from patient to patient. It is important to note, that often we find a higher degree of variation between normal rats of identical breed, matched for age and sex. Therefore, overall, patient tissue samples do not have as much variation was we originally suspected.

5. The Discussion was shortened as suggested by both this reviewer and reviewer #1.

We hope that we have adequately addressed the points raised by the reviewing team. We appreciate their time as well as that of the Editors. In our opinion, their input has significantly improved the quality of the manuscript.

As you requested, we have enclosed a copy of this cover letter, four copies of the revised manuscript with original illustrations, one copy clearly marked in red where revisions have been made (marked on the cover page as "RED"), one clean copy of the previous version of the manuscript and a diskette of the manuscript.

We look forward to hearing from you soon.

Sincerely,

Robert H. Getzenberg, Ph.D.
Prostate and Urologic Cancer Center
Assistant Professor of Pathology, Surgery,
Medicine and Pharmacology

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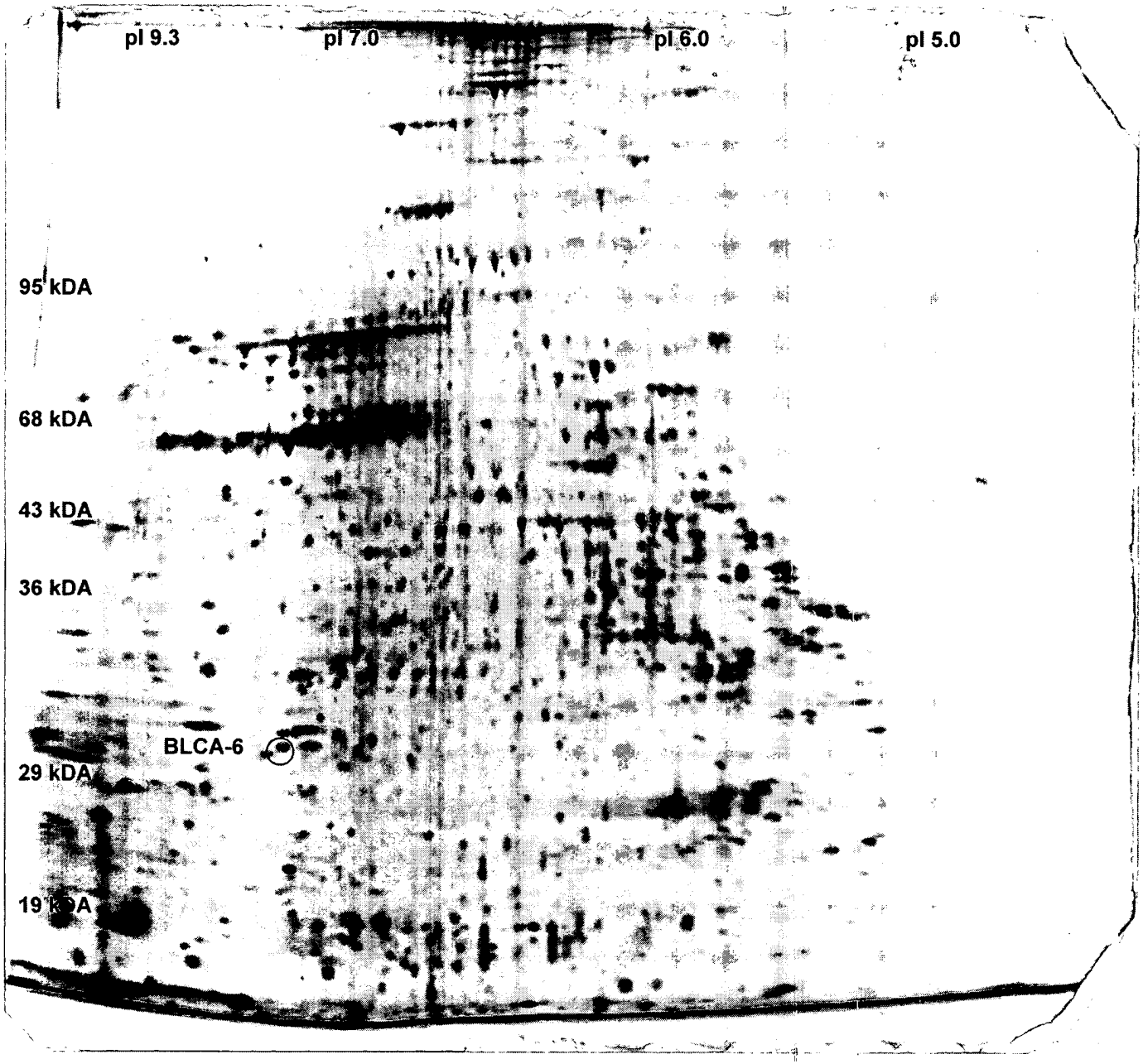
APPENDIX C


pI 9.3
pI 7.0
pI 6.0
pI 5.0
95 kDA
68 kDA
43 kDA
36 kDA
BLCA-6
29 kDA
19 kDA